Intrinsic subgroups or individual biomarkers for predicting outcome of metastatic breast cancer?

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Biomarker analysis of tumors from primary breast cancer patients has been commonplace for many years with analysis of estrogen receptor (ER) and HER2 status being mandatory for guiding therapy with anti-endocrine or anti-HER2 agents. As well as guiding adjuvant therapy these same markers, but few others so far, are used to guide treatment selection for metastatic disease; although they are increasingly often measured in metastatic biopsies their assessment in primary tumors remains the most frequent approach. Over recent years many multiparameter molecular tests have become available for breast cancer management. These are mainly aimed at establishing the risk of recurrence of patients with ER+ve disease who are due to receive standard endocrine therapy. Based on the prognosis of these patients, judgements can be made of the likely value of chemotherapy to improve that prognosis further. To date these prognostic tests have had no applicability in metastatic disease.

One of the multiparameter tests, derives from the global gene expression profiling work of Perou and colleagues about 15 years ago and is used to identify the so-called intrinsic subtypes of breast cancer[1,2]. The observation that the defined subtypes had prognostic significance[2] drove the interest in their application in breast cancer management. Through a number of iterations the subtypes, when assessed using RNA profiling, are now most commonly based on the PAM-50 classifier of 50 genes that can be used to characterise tissue that has been subject to routine formalin fixation and paraffin embedding[3]. The test is generally used to identify five major subtypes, Luminal A, Luminal B, HER2-enriched (HER2E), Basal-like or Normal-like, according to the correlation of the

expression of the 50 genes with an archetypal expression profile for that subtype.

An important aspect of the subtyping that appears to be poorly appreciated is that subtype is declared as the closest fit among the subtypes no matter how close or distant that fit is or how similar the fit is to a second subtype. In a recent study in which paired biopsies were available from 2 sets of untreated ER+ tumors, we noted a discordance of between 15 and 20% in the allocation of the subtype to Luminal A or B depending on which of the two biopsies was evaluated[4]. Unsurprisingly, this discordance was more apparent when the correlation of the samples was closely similar with the archetypal Luminal A or B subtype. This issue is not exclusive to the luminal subtypes, although the Basal-like subtype is generally clearly distinct.

In the article by Prat et al in the current issue[5], the authors took the unusual approach of assessing the clinical importance of intrinsic subtyping of tumors from postmenopausal patients with advanced hormone receptor positive breast cancer that had previously been untreated in the metastatic context. The patients had participated in a randomized clinical trial of letrozole with or without lapatinib (study EGF30008) that was unusual in that it included both HER2+ve and HER2-ve patients despite lapatinib being widely considered as a HER2-directed therapy[6]. Successful intrinsic subtyping was achieved in 821 tumor samples from the 1286 patients in the trial. In the original report of the trial[5], median progression-free survival (PFS) was increased from 3.0 months to 8.2

months by the addition of lapatinib in the 219 patients with HER2+ve disease but there was no improvement in PFS in the 952 patients with HER2-ve disease.

The current report[5] focuses largely on the prognostic importance of the subtyping in the HER2-ve population (a total of 644 patients in this substudy). As expected the majority (82%) were found to be Luminal (52% A and 30% B) in that population. Only 3% were identified as HER2E and 3% as Basal-like with 12% being called Normal-like. Intrinsic subtype (in most cases based on the phenotype of the primary tumor) made a strong contribution to the Cox multivariable model for both PFS and overall survival (OS) with the hazard ratios for Luminal B versus Luminal A being 1.47 and 1.57, respectively. The features that most obviously distinguish Luminal B from Luminal A tumors are higher proliferation and greater frequency of progesterone receptor (PgR)-negativity. This has led to the proliferation marker Ki67 and/or PgR being the most frequently used markers to create immunohistochemical surrogates for separating Luminal A from B subtypes among ER+ve/HER2-ve tumors[7,8]. As noted by Prat et al[5] several earlier publications identified relationships between Ki67 and/or PgR expression in primary tumors and PFS after first line metastatic treatment[9-11]. It is not clear from the paper by Prat et al[5] whether features other than the expression of proliferation-related genes or of PgR are implicated in this relationship with PFS.

Another key finding in the article by Prat et al[5] is that in the small subgroup of patients with HER2E/HER2-ve tumors outcome was exceptionally poor: median PFS was only 4.7 months (95% CI: 2.7-10.8) and OS 16 months (95% CI: 10-NA)

which were 2.9-fold and 2.5-fold poorer, respectively, than in patients carrying Luminal A tumors. Of particular note, however, the HER2E/ HER2-ve tumors showed significant benefit from lapatinib treatment for which the interaction test was significant (univariate P=0.02, multivariate P=0.006) even though there were only 16 patients of this phenotype in the study. Median PFS was still only 6.5 months in the group receiving lapatinib with letrozole but this compares with 2.6 months for those receiving letrozole alone. None of the other intrinsic subgroups of HER2-ve tumors showed benefit from lapatinib

Any interpretation of this result requires a greater understanding of what types of tumor constitute the HER2E group particularly what types of HER2-ve tumors. In the original studies by Perou and colleagues, which included ER-ve as well as ER+ve tumors[1,2], it was notable that ER+HER2+ tumors tended to segregate to the Luminal subtypes rather than HER2E. The Prat et al study deals with only ER+ve cases and this poses challenges to the normalization of data to allow accurate subtyping. Consistent with the earlier studies, only 29% of the HER2-positive tumors were classified as HER2E with similar numbers being classified as Luminal A (27%) or Luminal B (29%). SO there are large numbers of cases that might be considered as enriched for HER2 expression but are not subtyped as HER2E.

But why would some ER+ve/HER2-ve tumors be classified as HER2E when median levels of HER2 expression in them were no higher than in Luminal A HER2-ve tumors? What are the molecular features that lead to such tumors being described as HER2E if not HER2 itself? And why would they benefit from

added lapatinib? Examination of the heatmap showing the expression of genes for the tumors in the study (eFigure 2) is not illuminating: some of the HER2E/HER2-ve tumors cluster as part of an otherwise HER2E/HER2+ve group but most do not form a discrete group and are widely distributed across hierarchical clustering tree.

Prat et al comment that the efficacy of lapatinib in this group might be due to inhibition of EGFR rather than HER2: despite lapatinib being regarded largely as a HER2-targeted agent nowadays it also has activity against EGFR[12] and this was part of the rationale behind including HER2-ve cases in the EGF30008 study[6]. This was also part of reasoning for the inclusion of both HER2-positive and negative cases in a short-term presurgical study of lapatinib in patients with early breast cancer conducted by our group[13]. In this we observed 27% suppression of the proliferation marker Ki67 in the HER2-ve group (n=72) which was statistically significant although less than that in the HER2+ve group (46% reduction, n=19). EGFR expression did not correlate with the antiproliferative response in the HER2-ve patients but mRNA levels of HER3 did. The HER3 expression also correlated with higher HER2 transcript levels. Coombes et al[14] reported that 4/41 patients with HER2-ve breast cancer showed a >50% reduction in Ki67 in a similar study of short-term treatment with lapatinib: all four were HER3-positive. Heterodimerization of between HER3 and HER2 is known to enhance the kinase activity of HER2 (ref 15) and provides a plausible, though unproven, explanation for the impact of lapatinib in these HER2-ve tumors. HER3 is not included in the 50 genes of the PAM50 used for intrinsic subtyping so further interrogation of the molecular data from the Prat study

would be needed to determine if this interaction could similarly explain response in their HER2E/HER2-negative cases.

The novel study by Prat et al[5] of intrinsic subtyping in relation to progression of metastatic disease provided several provocative results. As with most other studies linking intrinsic subtype to clinical outcome, trying to understand the basis of the relationships inevitably leads us to try to disentangle the individual features that describe or are otherwise associated with the subtypes.

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